Parasitism of Ectomycorrhizae of Pine by Lance Nematode

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Abstract. Ectomycorrhizae were formed in aseptic culture on roots of *Pinus taeda* seedlings with *Pisolithus tinctorius* and in open pot culture on roots of *Pinus echinata* seedlings with *Thelephora terrestris*. Mycorrhizal and nonmycorrhizal roots on intact seedlings were inoculated with either 100 lance nematodes, *Hoplolaimus galeatus*, per individual glass cylinder or 6,000 per pot in a growth room equipped with electronic filters to remove airborne fungal spores. Irrespective of pine host, mycorrhizal fungus, or method used to form ectomycorrhizae, both male and female nematodes penetrated the fungal mantle of mycorrhizae. The nematodes readily parasitized cortical cells of roots morphologically altered by the fungal symbionts. Penetration of ectomycorrhizae by nematodes may allow entry of other root pathogens and also may lessen or destroy the beneficial physiological activity of ectomycorrhizae. **Forest Sci. 17:31–34.**

Additional key words. *Hoplolaimus galeatus, Pinus, Pisolithus tinctorius, Thelephora terrestris,* mycorrhizae.

THE LANCE nematode, *Hoplolaimus galeatus* Cobb, is commonly found in soils supporting pines in the southern United States (Ruehle 1964). It is pathogenic on slash (*Pinus elliottii* var. *elliottii* Engelm.) and loblolly (*P. taeda* L.) pines (Ruehle and Sasser 1962). It is also found associated with the roots of shortleaf pine (*P. echinata* Mill.) exhibiting littleleaf symptoms (Ruehle 1962), and it feeds and reproduces on the feeder roots of seedlings in the greenhouse (Ruehle 1969). Unaccountably, several recent studies by the senior author yielded inconclusive results about the effects of this nematode on the growth of shortleaf pine seedlings. There was a consistent high degree of variance in seedling growth within treatments, leading to the hypothesis that some uncontrolled factor was confounding the results.

Nematodes parasitizing pine trees limit themselves to the fine feeder roots (Ruehle and Sasser 1962), the same roots typically infected by ectomycorrhizal fungi. Initial colonization of soil by these fungi in greenhouse tests is erratic. The degree of mycorrhizal development on any particular seedling may also vary considerably (Marx and Bryan 1969, Marx *et al.* 1970). Since colonization of sterilized soil by fungal symbionts and the subsequent degree of ectomycorrhizal development is variable, we concluded that mycorrhizal development could have been the uncontrolled factor confounding the results of earlier studies.

To evaluate mycorrhizal and nonmycorrhizal roots as feeding sites for nematodes, we investigated parasitism of lance nematodes on both. This report presents our results of tests employing precisely controlled conditions of sterility, temperature, and moisture.

Materials and Methods

Test 1. This experiment was conducted in a special plant-growth room equipped with filters to remove airborne fungal spores (Marx and Bryan 1969). *Thelephora terrestris* (Isolate 2) was grown for 5 months at 25°C in 2-liter jars containing 1 liter of vermiculite and peat moss moistened with modified Melin-Norkrans liquid medium (Marx 1969) at pH 5.5 (Marx and Zak 1965). This inoculum was then mixed 1:4 by volume with a triple-autoclaved soil mixture (2 clay:1 sand:1 loam by volume) and placed in fumigated clay pots, 10 cm diameter. Identical inoculum was autoclaved and mixed with autoclaved soil, as the control mixture.

Shortleaf pine seeds were surface sterilized (Marx and Davey 1969a) and planted in the soil mixtures. Pots were automatically watered daily with 100 ml of nonsterile tapwater, and each received 100 ml of Melin-Norkrans (MN) salt solution (Marx 1969) after 1 month. Seedlings were thinned to 4 per pot after 6 weeks. Basidiocarps of *T terrestris* developed on stems of many seedlings growing in infested soil during the third month, but were removed to eliminate contamination of the control seedlings.

After 4 months, seedlings growing in soil infested with active inoculum had typical *T. terrestris* ectomycorrhizae on approximately 50 percent of the feeder roots. Mycorrhizae

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were absent on the control seedlings. At this time, 6,000 lance nematodes were added to each of 12 pots of mycorrhizal and 12 pots of nonmycorrhizal pine seedlings. Twelve pots of each group served as controls. After 43 days, the seedlings were removed and their roots examined at 30× magnification. Lateral root segments (1 cm long) with nematodes protruding from surfaces of either ectomycorrhizae or short roots were fixed, dehydrated, embedded in hard Tissuemat paraffin, serially sectioned (8 μ), and stained in safranine-fast green (Marx and Davey 1969a) for histological examination. Other segments with nematodes protruding were fixed in acetone-alcohol (50:50 v/v) for 12 hr and cleared in saturated chloral hydrate solution (48 hr) and 30-percent hydrogen peroxide (2 hr). After 20 to 30 days in 5-percent acid fuchsin-lactophenol, the roots were transferred to clear lactophenol. After 10 days the roots were again transferred to clear lactophenol and examined.

Test 2. Mycorrhizal and nonmycorrhizal shortleaf pine seedlings were removed from the pots and the roots cleaned of all debris. The seedlings were placed in modified 150- by 25-mm plastic petri plates. Lateral roots with or without ectomycorrhizae were inserted in root-inoculation cylinders and the insertion slots were sealed with a paraffin-lanolin mixture. The cylinders were then filled with sand and the remainder of the root systems was covered with sand moistened with MN nutrient salt solution (Marx and Davey 1969a). Six mycorrhizal and 3 nonmycorrhizal seedlings were arranged in separate plastic petri plates each with 4 lateral root segments in root-inoculation cylinders. Two cylinders on each seedling received approximately 100 lance nematodes in 1 ml of water, and 2 cylinders received water only. The plastic petri plates were wrapped in aluminum foil, and the seedlings, with foliage protruding, were incubated in a growth chamber adjusted for 12 hr of light (25 K-lux) at 24°C and 12 hr of darkness at 16°C. After 9 days the lateral root segments were removed from the root-inoculation cylinders, cleaned, examined, and processed for sectioning and mounting whole as described for Test 1.

Test 3. Loblolly pine seedlings were grown aseptically in 2-liter jars for 10 weeks and then inoculated with *Pisolithus tinctorius* (Pers.) Coker & Couch (Marx and Davey 1969a). Several seedlings were not inoculated and served as nonmycorrhizal controls. After incubation for an additional 10 weeks in a greenhouse water bath (24°C), the jars were filled with water and shaken vigorously, and the seedlings were removed. The roots were cleaned and lateral root segments with and without ectomycorrhizae were placed in root-inoculation cylinders as described above. Twelve mycorrhizal and 8 nonmycorrhizal seedlings were used. Each seedling had 4 lateral root segments in glass cylinders: 3 cylinders per seedling were each inoculated with approximately 100 lance nematodes in 1 ml of water and 1 cylinder received only water. After 9 days' incubation in the growth chamber the roots were cleaned and processed for histological examination.

Results and Discussion

Irrespective of pine host, mycorrhizal fungus, or method used to form mycorrhizae, both male and female lance nematodes penetrated the fungal mantle of mycorrhizae and the epidermis of nonmycorrhizal short roots (Fig. 1A, 1B). Some nematodes entered and migrated through the cortex; others penetrated perpendicular to the root axis and appeared to remain partially embedded. Nematodes occurred singly at points of entry and also fed in groups. Common entrance points were at natural breaks in the root surface where short root initials erupt through the surface of the lateral roots. Nematodes also penetrated the tips of lateral roots, including the root cap and region of elongation.

Nematodes were commonly found in the cortex of mycorrhizae and in the cortex of lateral roots supporting mycorrhizae (Fig. 1C, 1D). On nonmycorrhizal seedlings, however, nematodes were more commonly confined to the cortex of the lateral roots and were rarely found in the short roots.

No generalized necrosis was found in invaded mycorrhizae and neither hypertrophy nor hyperplasia was associated with nematode feeding. However, in a few instances toxified cells, *i.e.*, those intensively stained with safranine, were found 1 to 2 cells in advance of certain nematode feeding sites in the cortex of both lateral roots and mycorrhizae.

Lance nematodes are not reported to parasitize fungi, and all previous work on this species by the senior author shows this nematode feeding and reproducing only on higher plants. But, we now find that this nematode readily parasitizes cortical tissues of pine

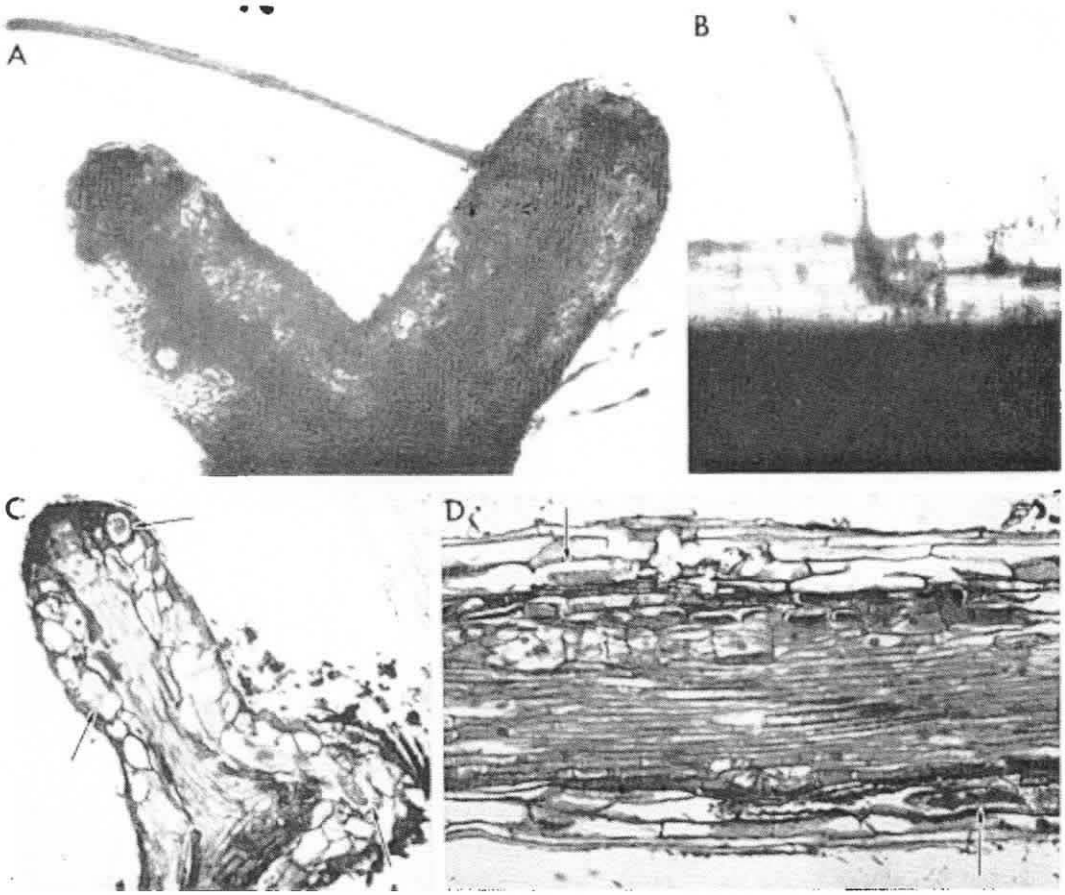

FIGURE 1. *Lance nematodes parasitizing ectomycorrhizae and lateral roots of pine. Cleared whole mounts show (A) female penetrating mycorrhiza and (B) male penetrating cortex of lateral root of shortleaf seedling. Longitudinal sections show (C) nematode migrating through cortex of shortleaf pine mycorrhizae and (D) through cortex of lateral root. A, B, D = 150×; C = 120×.*

roots morphologically altered by fungal symbionts.

The role of ectomycorrhizae as biological deterrents to pathogenic root infection, such as by *Phytophthora cinnamomi* Rands (Marx and Davey 1969a, 1969b), may be modified by nematode parasitism. Wounding of mycorrhizae caused by nematode penetration of the fungus mantle probably plays only a minor role, if any, in lowering resistance to fungus attack, because cells surrounded by the Hartig net still remain resistant (Marx and Davey 1969a, 1969b). However, physiological modification of such cortical tissue due to nematode feeding may alter this resistance. It is also possible that ectomycorrhizae parasitized by nematodes may not be as physiologically active as nonparasitized ectomycorrhizae and their benefit to the host thus reduced or lost.

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